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Usability of enzyme activity in estimation of forest soil quality

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Abstract

Taking into account the progressive degradation of soils it is important to assess their quality. Soil quality depends on a large number of physical, chemical, biological and biochemical properties. In the publications available, there are presented three approaches regarding the use of soil properties to estimate soil quality: (1) the use of individual properties, (2) the use of simple indexes and (3) the use of complex indexes derived from combinations of different properties. The purpose of this study was to investigate the possibility to use enzymes as indicators of forest soil quality. Experimental plots (43) were located in central Poland. The study was carried out in a number of diverse fresh forest sites. To assess the quality of forest soils dehydrogenase and urease activity and the degree of base saturation were used. One of the final conclusions point out that enzymatic activity indicates current site condition as well as the changes that occur in soil better than soil physical and chemical properties. In other words, in comparison to soil enzymatic activity, soil physico-chemical properties constitute a less sensitive indicator of soil changes.

Key words

soil enzymes, soil quality, forest soils, site index

Introduction

Both agricultural and forest soils are subject to improper use which leads to their degradation. Soil preparation, cultivation and use of heavy equipment may lead to soil fertility damage. Until now, most published papers have focused on evaluating the condition of agricultural soils and only a few have related to forest soil assessment. Forest soils are subject to fewer agricultural practices, yet forest vegetation impact on soil, and especially the impact of trees, differs in many ways from the impact of other groups of plants. In recent years, interest in soil quality has been stimulated by growing awareness of the fact that soil is an important component of the biosphere, that functions not only to produce food, wood and other forest resources, but it is also very important for maintaining local, regional and worldwide quality of the environment. Global efforts to evaluate soil are often connected with the concept of „soil quality". Gil-Sotres et al. (2005) found this term used in 1500 papers. This confirms the major interest in the issue of soil quality assessment. Papers published have shown that there are three approaches regarding the use of both general and specific biochemical pa-

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rameters to estimate soil quality: (1) the use of individual properties, (2) the use of simple indexes and (3) the use of complex indexes derived from combinations of different properties. Given that soil quality depends on physical, chemical, biological and biochemical properties of soil, changes in these properties must be taken into account while assessing changes in soil quality (Yakovchenko et al. 1996). Until now, physical and chemical properties have been considered in assessments of forest soils, whereas biological and biochemical properties have been ignored or treated marginally. In comparison with biological and biochemical properties, those physical and chemical constitute a less sensitive indicator of changes that occur in soil. Biological properties, as they are more sensitive and reflective of dynamic changes that occur in soil environment, can be used as a universal indicator that illustrates the results of forest soil exploitation. Soil and the processes that occur in it are crucial to maintain productivity of forest ecosystems. The assessment of soil quality is essential to monitor forest ecosystem stability. Soil properties that change over time slowly, cannot be used for soil quality assessment, especially in case of drastic changes in environment, In order to perform such an evaluation, properties that react quickly to environmental stress have to be used. Biological and biochemical soil properties react quickly to any changes, they are directly related to population and activity of soil microflora (microbial biomass, respiration etc.), as well as to the properties that are directly related to the amount and activity level of soil organic compounds (enzyme activity) (Trasar-Cepeda et al. 2008).

Individual soil biological and biochemical properties are not useful measures of soil quality (Skujins 1978; Nannipieri et al. 1990) because they vary both seasonally and spatially. It is, therefore, necessary to develop indexes based on a combination of soil properties that better reflect the effects of the major soil processes on soil quality (Dick 1994). The assessment of forest soils should be based on soil properties as well as the processes without which effective soil functioning would not be possible (Schoenholtz et al. 2000). According to Nannipieri et al. (2002), in order to assess soil one should use: the size of microbiological biomass, respiration, enzymatic activity, nitrogen mineralization and the number of fungi. Puglisi et al. (2006) so as to evaluate soil quality used soil enzyme activity (arylsulfatase, β-glucosidase, alkaline phosphatase, urease, invertase, dehydrogenase and fenoloksydase activity). Zornoza et al. (2007) created a forest soil quality index using physical, chemical and biochemical properties which are correlated with the content of nitrogen, organic carbon and microbial carbon. Trasar-Cepeda et al. (1998) showed that the soil quality can be expressed mathematically as a combination of several microbiological and biochemical properties (microbial biomass C, mineralized N, phosphomonoesterase, β-glucosidase and urease activity). Until now, only physical and chemical properties have been used for the assessment of Poland's lowland forest soil, while biological and biochemical properties have been ignored. Brożek (2007) created the Soil Index of the Site for the evaluation of forest soils quality. The Index contains the sum of the following indicators: content of fraction < 0.02 mm, content of base cations (Ca+K+Mg+Na), percentage of total nitrogen in first mineral horizon multiplied by 1/C:N and content of hydrogen ions determined as total acidity divided by fraction < 0.02 mm content. Januszek (2011) created the soil quality index (SEI_{DPU}) for mountain and upland forest soils, which is based on a simple formula combining soil dehydrogenase, urease and protease activities. Olszowska et al. (2005, 2007) created the Biological Indictaor of Soil Fertility. The indicator contains the sum of soil biological activity and the sum of base cations and base saturations.

In forestry, as in agriculture, the most important aim is to obtain the best yield. In forestry tree growth or the amount of wood harvested are traditional measures of soil productivity. Foresters usually define soil productivity as capacity of soil to produce biomass in a definite area in a definite period of time (Ford 1983).

The purpose of this study was to determine the enzymatic activity of soils with different trophism, forming different site conditions – from poor soils of coniferous forests through mesotrophic soils of mixed coniferous and mixed broadleaf forests to rich soils of broadleaf forest site. Another aim was to use the data obtained to develop an index of lowland sites quality. Next objective of the study was to determine a relationship between productivity (site index) of selected forest soils and their physiochemical and biochemical properties. This information could be useful for foresters, who would be able to plan species composition of tree stands

in order to prevent soil degradation and modification of soil properties.

Material and methods

Soils

The samples from forty-three research plots were collected from sites in Central Poland, in the Przedbórz and Smardzewice Forest Districts. Soil samples were collected during July 2007. Research plots (11) were covered with pine trees on the *podzols* and *arenosols* soils. The study also included 11 (*podzols*) stands – dominated with pine and spruce, and 11 (*brunic arenosols, stagnosols*) stands with mixed broadleaf forests (oak and pine). Other 10 (*cambisols)* stands were covered with oak trees. At each sample plot, a detailed description of soil profile was carried out, the samples were taken from each soil genetic horizon in order to perform basic evaluations of soil properties. The soil for determining enzyme activity was collected after removal of the litter layer. In all the cases, research samples were collected from 8 sub-stands of the upper soil horizon (0–10 cm), pooled in the field, transported to the laboratory and then sieved through a 2 mm sieve. The samples were stored at 4°C until further analysis.

Soil physical and chemical properties

Granulometric composition of the samples was determined following the aerometric method, their pH – with the potentiometric method (in water and 1M KCl), total nitrogen content – with Kjeldahl's method, organic carbon content with – Tiurin's method, including calculation of C/N ratio, the content of alkaline cations – in 1M ammonium acetate, by means of calculation of the degree of base saturation (V%), and available phosphorus – with the Bray- Kurtz method. Bulk density was determined using Kopecky's cylinder method.

Enzymatic activities

Dehydrogenase activity was marked with Lenhard's method according to the Casida procedure, expressed in triphenyl formazan milligrams (TFF) for 100g of soil after 24 hours. The method is known as „the TTC test", 3 per cent solution of triphenyltetrazolium chloride (TTC). Ethyl alcohol contaminated with methanol was used for washing out formazan gathered in soil (Alef and Nannipieri 1995). Protease activity was marked with the Hoffman and Teicher method (Haziev 1976), with application of 2% solution of gelatin as substrate, and enzyme activity expressed in N-NH_2 mg for 100 g of soil during 20 hours. Urease activity was marked with the use of the method Tabatabai and Bremner (1972 in: Alef and Nannipieri 1995) and enzyme activity expressed in μg N-NH_4 for 1 g of soil during 2 hours. The activity of β-Glucosidase was assessed with the method of Eivazi and Tabatabai (1990), using the substrate *para*-nitrophenyl- β-D-glucopyranoside (*p*NPG). By means of soil bulk density, the enzymatic activity was calculated for 1 dm^3 of soil.

Site index

In this study, the site index – determined on the basis of the model of stand top height growth – was used for productivity estimations. For pine stand according to Bruchwald and Kliczkowska (2000):

$$B = \frac{h}{A}$$

$$A = \left(\frac{w}{30 + 0,278675 w^{1,2}} \right)^{0,00007 w^2 - 0,0005 w + 1,8}$$

where:

w – tree age,
h – tree height at age w,
B – height growth rate,
A – standardized tree height.

For oak stand according to Bruchwalda et al. (1996):

$$B_{100} = \frac{H_{100}}{A}$$

$$A = \left[\frac{1}{7,3046019} \right] \times \left[\frac{w}{(7 + 0,3 \times w)} \right]^2$$

where:

w – tree age,
h_{100} – tree height at age w,
B_{100} – height growth rate,
A – standardized tree height.

The diameter and height of all trees were measured for each study area. The age was determined by Pressler bit.

Statistical analysis

Statistical analysis of data was performed using Statistica 9 software, differences between the means samples were tested with the Kruskal-Wallis test. In order to determine which soil properties differentiate between these groups of research site, discriminant analysis was used.

Results

Soil physical and chemical properties

The soils under pine forest and mixed coniferous forests (pine and spruce) were of similar texture (sand and loamy sand). The soil under mixed broadleaf forests (oak and pine) and under oak forests contained more clay (loamy sand, sandy loam and loam). In general, the soils tested were acidic, the soils of pine forest were characterized by the lowest pH (average pH in H_2O 3.68, pH in KCl 2.84). The highest pH was noted in oak forests soils (average pH in H_2O 4.47, pH in KCl 3.72 , tab. 1). Large differences in N and C content in the latter site soil were recorded because of different rates of decomposition of organic matter. Average content of organic carbon was 23.12% in pine forest soils and 1.85% in the soil under oak forests. The highest N content was noted in the soil under coniferous forest (0.81%), and the lowest – under broadleaf forest (0.13%). The highest mean V% was noted in the soil under broadleaf forest site (77,8%), and the lowest – under coniferous forest (12,8%).

Enzymatic activities

The activity of dehydrogenase varied among the 43 soils observed and was from 3.47 to 81.84 μmol TFF $kg^{-1}h^{-1}$ (tab. 2). The soils of pine forest were characterized by the lowest dehydrogenase activity (12.70 μmol TFF $kg^{-1}h^{-1}$ on average), whereas the highest activity was noted in

Tab. 1. Mean values and range of variation of main soil properties

Soil properties	Coniferous forest site		Mixed coniferous forest site		Mixed broadleaf forest site		Broadleaf forest site	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
pH H_2O	3.68	3.30–4.08	3.99	3.54–4.70	4.12	3.68–4.69	4.47	4.03–4.98
pH KCl	2.84	2.60–3.32	3.12	2.80–3.50	3.41	2.98–4.14	3.72	3.41–4.39
Total C(%)	23.12	7.62–40.00	17.79	10.62–27.65	5.63	2.10–17.86	1.85	0.91–2.62
Total N(%)	0.81	0.32–1.42	0.68	0.48–0.97	0.31	0.11–0.78	0.13	0.07–0.21
C/N	28	23–34	26	19–36	17	14–23	14	8–19
Available P	37.1	15.9–59.9	50.9	34.9–87.6	50.1	3.1–122.5	44	5.5–133.1
V%	12.8	7.3–18.5	28.8	9.8–74.1	40.2	6.5–71.3	77.8	54.2–98.3
Sand (%)	97	91–100	89	65–100	78	63–99	54	6–79
Clay (%)	1	1–1	3	1–15	6	1–16	16	2–30

V% – degree of base saturation

Tab. 2. Mean values and range of variation of enzymatic activities

Soil properties	Coniferous forest site		Mixed coniferous forest site		Mixed broadleaf forest site		Broadleaf forest site	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Dehydrogenase[a]	12.70	3.47–24.39	25.04	9.95–52.61	30.83	11.75–42.45	46.51	23.38–81.84
Urease[b]	0.31	0.18–0.61	0.34	0.14–0.64	0.38	0.13–0.51	0.43	0.31–0.69
Protease[c]	8.23	5.42–12.51	8.04	4.63–12.52	7.59	4.78–9.58	6.47	5.23–8.29
β-glucosidase[d]	0.17	0.04–1.16	0.05	0.004–0.13	0.36	0.01–1.68	0.04	0.003–0.09

a – μmol TFF $kg^{-1}h^{-1}$; b – mmol N-NH_4 $kg^{-1}h^{-1}$; c – mmol N-NH_2 kg^{-1} h^{-1}; d – μmol pNP $kg^{-1}h^{-1}$.

Tab. 3. Correlations between enzyme activities and physico-chemical properties of soil

Soil properties		pH H_2O	pH KCl	Total C (%)	Total N (%)	C/N
Enzymatic activity calculated over to 1 kg of soil	Dehydrogenase	0.71^{***}	0.74^{***}	-0.59^{***}	-0.55^{***}	-0.62^{***}
	Urease	0.38^{*}	0.41	–0.19	–0.19	–0.32
	Protease	–0.28	–0.28	0.36^{*}	0.38	0.26
	β-glucosidase	–0.27	–0.13	0.07	0.06	0.02
Enzymatic activity calculated over to 1 dm^3 of soil	Dehydrogenase	0.67^{***}	0.73^{***}	-0.72^{***}	-0.72^{***}	-0.71^{***}
	Urease	0.57^{***}	0.69^{***}	-0.73^{***}	-0.72^{***}	-0.76^{***}
	Protease	-0.49^{**}	-0.59^{***}	-0.71^{***}	-0.69^{***}	-0.79^{***}
	β-glucosidase	0.02	0.03	0.13	0.10	0.18

*** – significant at α = 0.001; ** – significant at α = 0.01; * – significant at α = 0.05.

oak forest soils (46.51 μmol TFF $kg^{-1}h^{-1}$ on average). The activity of dehydrogenase was most strongly correlated with pH in KCl (r = 0,74), but also strongly negatively correlated with C (r = –0,59) (tab. 3).

The highest urease activity was noted in oak forest soils (on average 0.43 mmol $N\text{-}NH_4$ $kg^{-1}h^{-1}$) (tab. 2). The mean values of urease activity in the soils under mixed coniferous forests and mixed broadleaf forests (0.34 and 0.38 mmol $N\text{-}NH_4$ kg^{-1} h^{-1}) were higher than those in the soils under pine forest (0.31 mmol $N\text{-}NH_4$ $kg^{-1}h^{-1}$). The activity of urease calculated over to 1 dm^3 of soil was most strongly negatively correlated with concentrations of N and C (r = –0.73 and r = – 0.72) (tab. 3).

The results of protease activity showed opposite order with regard to the activity of dehydrogenases and urease. The soils of pine forest were characterized by the highest protease activity (8.23 mmol $N\text{-}NH_2$ $kg^{-1}h^{-1}$ on average), and the lowest activity was noted in oak forest soils (6.47 mmol $N\text{-}NH_2$ $kg^{-1}h^{-1}$ on average) (tab. 2). The mean values of protease activity in the soils under mixed coniferous forests and mixed broadleaf forests (8.04 and 7.59 mmol $N\text{-}NH_2$ $kg^{-1}h^{-1}$, respectively) were higher than in the soils under oak forests. The activity of protease calculated over to 1 dm^3 of soil was most strongly negatively correlated with concentrations of C and N (r = –0.72 and r = –0.69) (tab. 3).

The highest activity of β-glucosiadase was observed in the soils under stands of oak with high proportion of pine, and the lowest – in the soils under oak trees. The enzymatic activity was not correlated with physico-chemical properties of the soils examined.

As a result of discriminant analysis there was found which properties of soil best specified soil quality: dehydrogenases and urease activity in the humus horizon calculated over to 1 dm^3 of soil and the degree of base saturation in the transition level to parental material. The canonical discriminant function took the following form:

$$W = ADh + AU + V\%$$

where:

W – forest soil quality index,

ADh – dehydrogenases activity in the humus horizon calculated over to 1 dm^3 of soil,

AU – urease activity in the humus horizon calculated over to 1 dm^3 of soil,

V% – degree of base saturation in the transition level to parental material

Table 4 shows the soil quality index calculated for different forest sites.

Tab. 4. Forest soil quality index

Forest sites	Forest soil quality	
	Mean	Range
Coniferous forest site	30	17–40
Mixed coniferous forest site	76	41–157
Mixed broadleaf forest site	268	179–344
Broadleaf forest site	458	259–727

Site index

The site index – estimated on the basis of the model of top height growth of pine stand was the lowest in coniferous forest site (21.51). The highest value (31.80) was noted in mixed broadleaf forest site (fig. 1).

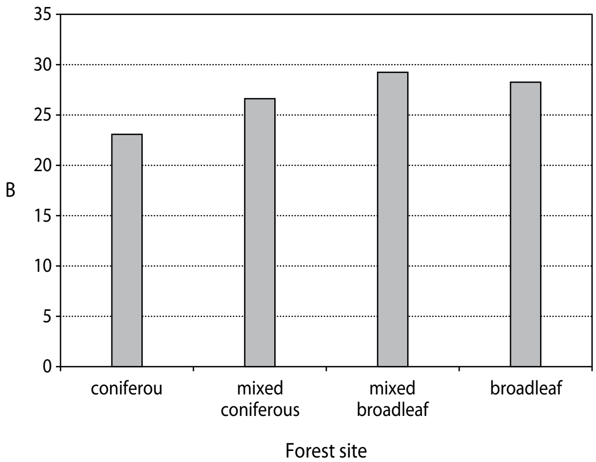

Fig. 1. Relationships between the site index of pine stands (B) and forest site type

The site index estimated on the basis of the model of top height growth of oak stand was the lowest in mixed coniferous forest site (20.95). The highest value (24.91) was noted in broadleaf forest site (fig.2).

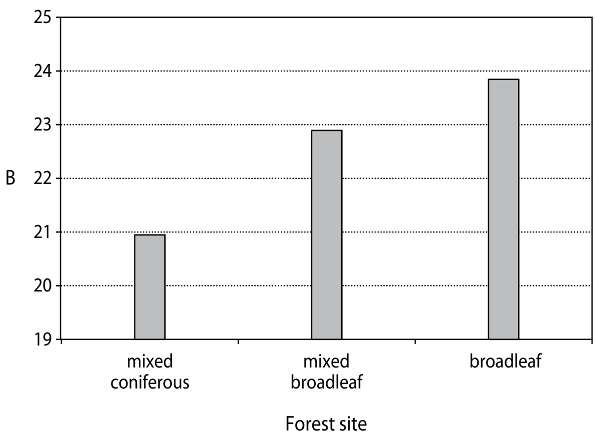

Fig. 2. Relationships between the site index of oak stands (B) and forest site type

The site index was correlated with enzyme activity. The site index of pine stand was strongly correlated with dehydrogenase activity in the humus horizon expressed in units of weight (r = 0.66) (tab. 5). Similar correlations were obtained between the site index of pine stands and urease and protease activity in the humus horizon calculated over to 1 dm^3 of soil (r = 0.51, r = 0.52) (tab. 5). The site index of oak stand was strongly correlated with urease activity calculated over to 1 dm^3 of soil (r = 0.95). Soil quality index was correlated with the site index of pine and oak stand (r = 0.62, r = 0.79) (tab. 5).

Tab. 5. Correlations between site indexes and soil quality index and enzymes activity

Site index	W	ADh	AU	AP
Pine	0.62***	0.66***	0.51**	0.52**
Oak	0.79*	0.66	0.89*	0.37

W – soil quality index, ADh – dehydrogenase activity in humus horizon expressed in units of weight, AU – urease activity in the humus horizon calculated over to 1 dm^3 of soil, AP – protease activity in the humus horizon calculated over to 1 dm^3 of soil, *** – significant at α = 0.001; ** – significant at α = 0.01; * – significant at α=0.05

Discussion of results

Dick (1994) believes that soil enzyme activity may be a good indicator of soil quality because determination of the enzymatic activity is relatively simple and quick, and enzymes react quickly to changes in the environment. In addition, the enzymatic activity in soil plays an important role in catalyze reactions necessary for life processes of soil microorganisms, decomposition of organic residue as well as circulation of nutrients in the creation of organic matter and soil structure. Enzymatic processes are closely related to soil quality, they participate in the processing of unavailable forms of nutrients readily assimilated by plants (Sinsabaugh et al. 1994). Single biological and biochemical properties should not be used to assess soil quality because they are subject to seasonal and spatial changes (Nannipieri et al. 1990; Nannipieri 1994). It is, therefore, necessary to create indexes which are a combination of several soil properties, which more fully reflect fertility and quality of soil. The results obtained indicate that physical and chemical properties are less distinguishable in the tested soils in comparison to the activity of enzymes. The soils under coniferous forest and mixed coniferous forest had similar physical and chemical properties, such as texture, pH in H_2O and KCl, and C.N ratio (tab. 1). Urease and dehydrogenase activities significantly differed and separated the soils under coniferous forest and mixed coniferous forest (tab. 2).

Taking into account the opinions of the authors quoted above, in order to build the index of forest soil

quality there were used the activities of dehydrogenases and urease in the humus horizon calculated over to 1 dm^3 of soil and the degree of base saturation in the transition level to parental material. The formula of this index uses biochemical and chemical properties of soil which were selected using statistical methods. Many researchers limited the number of properties on which they based their rates, treating physical and chemical properties as less important in assessing soil quality (Stefanic et al. 1984; Nortcliff 2002; Gil-Sotres et al. 2005). Gil-Sotres et al. (2005) believe that creating a universal tool to assess soil quality is very difficult because climatic conditions (temperature, precipitation, etc.) are very diverse. In this paper the authors use the activity of two enzymes (dehydrogenase and urease), which are cited in literature as a good tool to assess soil quality Stefanic et al. (1984), Beck (1984), Myśków et al. (1996), Kucharski (1997), Januszek (1999), Koper and Piotrowska (2003), Lasota (2005), Puglisi et al. (2006).

The activity of urease as well as dehydrogenase reacts promptly to changes in the use of soil (Pascual et al. 1999; Saviozzi et al. 2001; Gil-Sotres et al. 2005). Kandeler and Eder (1993) suggest that urease activity could be used as an indicator of changes in soil. Kucharski (1997), Januszek (1999), Gil-Sotres et al. (2005), Puglisi et al. (2006), Zornoza et al. (2007) propose urease activity as one of the components of biological indicators to assess soils. Many authors mention β-glucosidase as a useful parameter for determining soil quality because it negatively reacts to agricultural operations, although organic fertilization increases its activity (Bandick and Dick 1999; Pascual et al. 1999). Eivazi and Tabatabai (1990) reported a positive correlation between β-glucosidase activity and organic carbon content and negative correlation with pH. The close relationship between β-glucosidase activity and soil properties (microbial biomass, organic matter, soil texture) suggest that β-glucosidase activity may provide information about soil quality and it can play an important role in monitoring biological soil quality (Turner et al. 2002). Trasar-Cepeda et al. (2000) concluded that the activity of β-glucosidase in addition to urease activity as well as the activity of phosphomonoesterases and dehydrogenases can be used to assess soil degradation. Rodríguez-Loinez et al. (2007) successfully used β-glucosidase activity to assess the quality of arable and grassland soils. These studies did not confirm the above-described relation. The β-glucosidase activity showed the lowest correlation with physical and chemical properties of the soils observed. This suggests that β-glucosidase activity is not a good indicator to assess forest soils.

Sorption capacities of soils determine storage of nutrients, immobilization of toxic elements responsible for water retention in soil and circulation of trace elements. In our study, one component of the formula for assessing soil quality is the degree of base saturation in the transition level to parental material. The fertility of the parent material should be appreciated because together with soil moisture and climatic conditions it jointly determines soil-forming process, soil fertility and its suitability for silviculture. Particularly, in the case of mountain forest soils, indicators of their potential for production and suitability for forestry and farming. Therefore, the quality index developed includes the degree of soil saturation with alkaline cations in the transition level to parental material. Lasota (2005) believes that deeper soil levels cannot be omitted in the calculation of soil quality index because they often constitute a kind of "reservoir" of nutrients which co-decide about the quality of forest soils.

Quantitative assessment of the quality of forest soils is difficult. Research has been conducted in this area for many years. In agriculture, the role of such index is usually expressed by crop yields. The whole produced biomass is also often used as an indicator. The use of these methods is limited in the case of trees,. In Polish forest practice the tree height relative to the species and age is most often used as an indicator of site quality, assuming productivity due to the quality of habitat. Carmean (1975) believes that the quality of site may be expressed by the height of trees of a certain age. Schoenholtz et al. (2000) believe that the quality of soil is a part of site quality and should be expressed by the growth of trees or biomass produced. The results obtained indicate a direct relationship between the enzymatic activity and productivity of soil, which is related to the quality of the site. The results confirm the usefulness of soil enzymatic activity to assess the quality of soil. The correlation between the site index and soil quality index was found. A similar correlation between the quality of soil and its productivity was described by Skinner et al. (1999).

Conclusions

- To assess the quality of forest soils dehydrogenase and urease activity and soil base saturation in the transition level to parental material were used.
- The activity of dehydrogenase and urease indicates site current condition along with the changes that occur in soil better than soil physico-chemical properties.
- The activity of β-glucosidase showed the weakest correlation with physico-chemical properties of soils. This suggests that β-glucosidase activity is not a good indicator to assess forest soils.
- The correlation between the site index and soil quality index obtained confirm the usefulness of the enzymatic activity for assessments of soil quality

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